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Note

Determination of oxytetracycline, sulphamethazine and sulphamethoxypyridazine in feed premixes

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Sulphonamides are frequently used as antimicrobial agents, often in association with antibiotics to promote growth and prevent disease in animals. The simultaneous determination of various sulphonamides has been investigated by high-performance liquid chromatography (HPLC) using both normal-phase[1] and reversed-phase modes[2–7]. HPLC is suitable for the determination of sulphonamides in biological fluids[1,3–6], pharmaceutical preparations[2] and feeds and feed premixes[7]. The determination of tetracyclines has been also achieved by reversed-phase HPLC[8–10].

This paper describes a rapid reversed-phase HPLC method for the simultaneous determination of the sulphonamides sulphamethazine and sulphamethoxypyridazine in association with oxytetracycline in feed premixes.

EXPERIMENTAL

Instrumentation

An LDC high-performance liquid chromatograph was purchased from Sopares (France) and equipped with a Constametric III pump, a Valco 7000 PSI injector and a Spectromonitor III UV spectrophotometer set at 254 nm and 0.5 a.u.f.s.

Peaks areas were measured and printed with an LDC/Milton Roy CI/10 integrator.

Reagents and chemicals

Sulphamethazine sodium, sulphamethoxypyridazine sodium and oxytetracycline hydrochloride were purchased from Sica-Deltavit (France). Acetonitrile, sulphuric acid and dimethylformamide (DMF) were obtained from Carlo Erba (Italy).

Procedure

Chromatography was carried out on a stainless-steel column (20 × 0.47 cm I.D.) of Spherisorb ODS Hichrom (particle size 5 μm) at room temperature.

The separation of sulphamethazine sodium, sulphamethoxypyridazine sodium

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and oxytetracycline hydrochloride was first tested by isocratic elution with acetonitrile–0.05 *N* sulphuric acid. Subsequently, in order to obtain a better separation, DMF was incorporated at different concentrations. The flow-rate was 1 ml/min.

Standard solutions of sulphamethazine sodium, sulphamethoxypyridazine sodium and oxytetracycline hydrochloride prepared at concentrations of 200, 150 and 300 μg/ml, respectively, in 0.05 *N* hydrochloric acid. A mixture was also prepared containing the same concentrations.

Volumes of 25 μl of each sample were injected into the chromatograph.

RESULTS AND DISCUSSION

The different solvent systems used and the corresponding retention times of the antimicrobial agents are reported in Table I.

TABLE I

SOLVENT SYSTEMS AND RETENTION TIMES FOR THREE ANTIMICROBIAL AGENTS

Solvent	*Component (%, v/v)*			*Retention time*		
	Acetonitrile	*0.05 N Sulphuric acid*	*Dimethyl-formamide*	*Sulphamethazine sodium*	*Sulphamethoxy-pyridazine sodium*	*Oxytetracycline hydrochloride*
A	21	79	0	5 min 30 sec	7 min	9 min 15 sec
B	24	76	0	3 min 30 sec	4 min 45 sec	6 min 15 sec
C	27.5	72.5	0	3 min	3 min 40 sec	4 min 30 sec
D	20.5	76.5	3	5 min	6 min 30 sec	7 min 45 sec
E	19.5	74	6.5	4 min 10 sec	5 min 40 sec	6 min 55 sec

When the solvent systems without dimethylformamide (DMF) were used, the retention times of the compounds decreased with increase in the acetonitrile concentration. Unfortunately, the resolution of oxytetracycline was poor at lower acetonitrile concentrations and, although it was partially improved by increasing the acetonitrile concentrated the three compounds were then not well separated (Fig. 1).

The addition of DMF to the solvent provided an acceptable resolution of oxytetracycline and also gave a good overall separation of all three compounds (Fig. 2). An increase in the concentration of DMF from 3 to 6.5% appreciably improved the resolution of oxytetracycline, but above 6.5% the separation of the three compounds was incomplete. Hence solvent system E (Table I), containing 6.5% of DMF, was selected for reproducibility studies.

The limits of detection were 20 ng for sulphamethazine sodium and sulphamethoxypyridazine sodium and 80 ng for oxytetracycline hydrochloride.

A linear relationship was found between the concentration of sulphamethazine sodium, sulphamethoxypyridazine and oxytetracycline injected and their peak-area ratios (from 100 to 30 000 ng injected, $R > 0.99$).

The reproducibility of the method was tested by six consecutive injections of the same sample (Table II). The good reproducibility obtained permits the direct

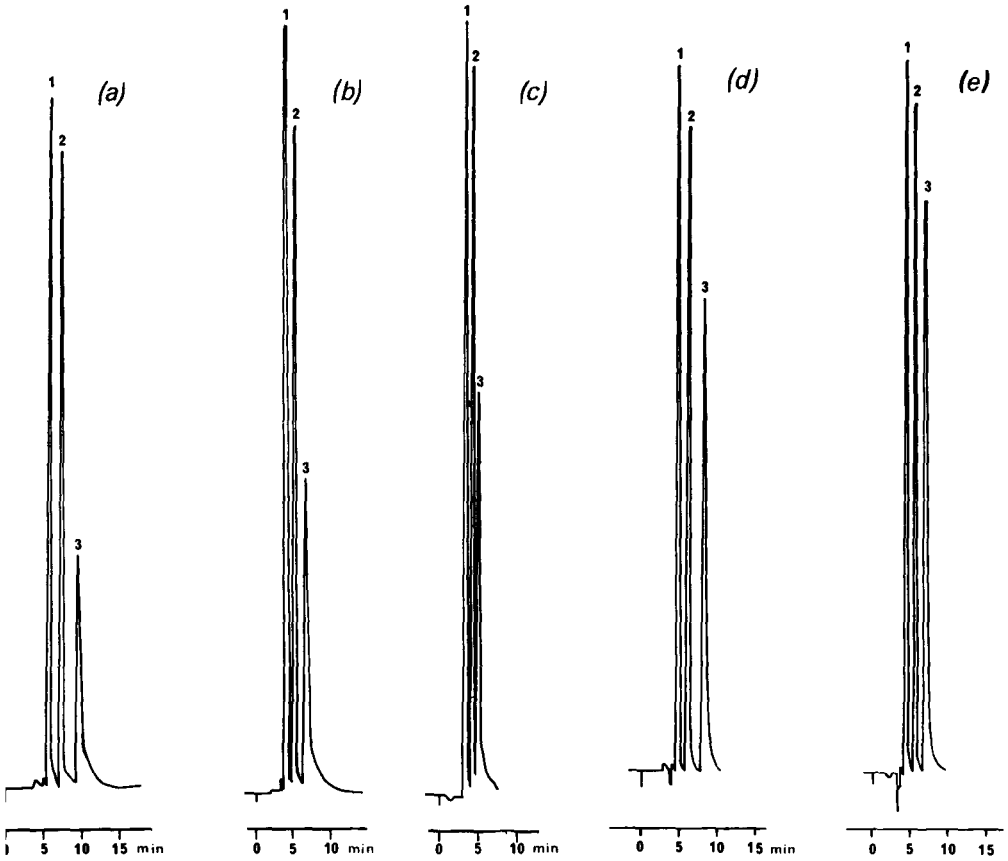

Fig. 1. HPLC separation and UV detection of sulphamethazine sodium (1), sulphamethoxypyridazine sodium (2) and oxytetracycline hydrochloride (3) in feed premixes. Conditions: column, Spherisorb ODS Hichrom; flow-rate, 1 ml/min; UV detection, 254 nm. Eluent: (a) acetonitrile–0.05 *N* sulphuric acid (21:79, v/v); (b) acetonitrile–0.05 *N* sulphuric acid (24:76, v/v); (c) acetonitrile–0.05 *N* sulphuric acid (27.5:72.5, v/v).

Fig. 2. HPLC separation and UV detection of sulphamethazine sodium (1), sulphamethoxypyridazine sodium (2) and oxytetracycline hydrochloride (3) in feed premixes. Conditions as in Fig. 1. Eluent: (d) acetonitrile–0.05 *N* sulphuric acid–DMF (20.5:76.5:3, v/v); (e) acetonitrile–0.05 *N* sulphuric acid–DMF (19.5:74:6.5, v/v).

TABLE II

PEAK AREAS

Peak areas found after six injections of 25-μl samples containing 5.000, 3.750 and 7.500 μg of sulphamethazine sodium, sulphamethoxypyridazine sodium and oxytetracycline hydrochloride, respectively.

Compound	*Peak areas*	*Mean peak area*	*Standard deviation*	*Coefficient of variation (%)*
Sulphamethazine sodium	215 000, 216 000, 214 500, 216 500, 216 000, 215 000	215 500	775	0.36
Sulphamethoxypyridazine	182 600, 184 000, 184 200, 183 400, 182 000, 182 500	183 120	886	0.48
Oxytetracycline hydrochloride	290 000, 288 000, 289 000, 292 000, 290 500, 289 500	289 830	1366	0.47

analysis of the three antibiotics without the addition of an internal standard.

The use of classical eluents does not allow the determination of oxytetracycline combined with sulphamethazine and sulphamethoxypyridazine. As demonstrated here, this problem was solved by the addition of DMF to the eluent system, without affecting the column efficiency.

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